A BRIEF REVIEW OF A SURVEY OF THE VARIANT PULLORUM PROBLEM

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Research workers in Canada have long been aware of a problem in pullorum disease control due to antigenic differences in strains of Salmonella pullorum. Younie[1] first called attention to this problem when, in 1941, he reported on outbreaks of pullorum disease in chicks from flocks negative to the standard agglutination test. A retest of parent stock detected only 11 reactors among some 10,000 birds. The isolation of a Salmonella pullorum variant was reported by Younie the same year. Several of the flocks from which this variant strain could be isolated were totally negative to the standard agglutination test. Extensive and painstaking experimental work with respect to this problem has since been carried on by the Canadian investigators and many valuable contributions have been added to the scientific literature. Among the Canadian investigators, other than Younie, who have reported studies in the problem of variant pullorum are Bond, Byrne, Gwatkin, and Wright.

Very little work has been published by investigators in the United States. Edwards and Bruner[2] have shown that the difference between standard and variant strains is essentially a form variation involving the XII antigenic factor. These investigators later reported[3] that approximately 1/3 of S. pullorum cultures isolated in Kentucky were variants. Wright[4] reported at the 1944 conference of this group that he had found variants among cultures examined from 14 of the 16 Northeastern Conference States. In investigations reported by the Bureau[5] in 1947, it was found that birds artificially infected with variant strains reacted to standard whole-blood plate antigens as well as they did to variant plate antigens.

Early in 1947 the U. S. Bureau of Animal Industry received reports from some of the States advising of pullorum disease breaks in chicks from parent stock negative to the agglutination test with the standard stained antigens, T. G. and K. These breaks could not be explained on the basis of hatchery or farm contamination. A retest of some of the parent stock with standard whole-blood antigens and with a commercially produced polyvalent antigen comprised of standard and variant strains frequently disclosed positive and suspicious reactions to the polyvalent antigen.

In October, 1947, veterinarians from the Bureau of Animal Industry were sent to the States of Indiana, Kentucky, Minnesota, and Ohio to observe, under field conditions, the pullorum testing of hatchery supply flocks in which variant pullorum had been reported. In each State birds were found that failed to react to T. G. and K antigens, but gave positive and suspicious reactions to antigens comprised of standard and variant strains. Accordingly, 117 of such birds were purchased and shipped to the U. S. Animal Disease Station at Beltsville, Maryland, for additional tests with standard and variant antigens and for postmortem examination. The present paper is a summary of the work done at Beltsville.

Tests and Postmortem Results on Variant Reactor Birds

The marked difference between the percent of reactors to the standard antigen and the percentage of reactors to the polyvalent antigens in 5 plate tests and 2 tube tests is shown in Table 1. The first test in the table shows the results of the field tests; the next 4 show the results of plate tests made at Beltsville. All plate tests were made with whole blood.

These tests are not all strictly comparable with one another since the number tested varied with each test. However, it will be noted that there is steady increase in the percentage of birds that react to the standard antigen in the first 4 plate tests. This is apparently due largely to changes in the reactions of individual birds. Thus, 8 of 24 birds in which S. pullorum was found were negative to standard antigen at first but reacted on later tests. This might be intrepreted to indicate that as the birds get older variant pullorum infected birds tend to react to standard antigens. Averaging all 5 plate tests, it may be noted that the percentage of reactors to the polyvalent antigen is more than 3 times the percentage positive to the standard antigen. In the tube tests the difference in the percentage of reactors to the two antigens is much less than in the plate tests, although even here nearly twice as many reacted to the polyvalent as to the standard antigen.

Thus far, 75 of 117 birds have been subjected to postmortem examination. These birds were sacrificed in 6 lots and 12 birds (lot 7) died, as shown in Table 2. S. pullorum was isolated from 26 birds. Of the 26 in which S. pullorum was found, 24 were negative to standard antigen and 2 were positive. All of the birds (26) were positive to polyvalent antigen on the field test. S. pullorum was not isolated from 49 of the 75 birds autopsied, but 1 revealed S. gallinarum. Of 16 pullorum cultures typed, 15 were variants and one was rough. The birds in lot 5 were all from one State, were very weak or negative reactors, and none showed pullorum lesions; S. pullorum was recovered from only 1 bird of the 17 in this lot. These birds were all questionable reactors and were selected by the use of a commercial polyvalent antigen by State pullorum testing crews. In lots 2 and 3 cultures were made from 8 organs of each bird -- the duodenum, ovary, liver, gall bladder, spleen, lungs, ceca, and pericardial sac. In the remaining lots, 12 organs were cultured -- ovary, oviduct, liver, kidney, spleen, lung, crop, proventriculus, pancreas, duodenum, gall bladder, and pericardial sac.

The frequency of isolation of S. pullorum from the various organs is shown in Table 3, which is self-explanatory.

Table 4 is a breakdown of Table 3. The fact that 42 percent of the total isolations were from the digestive tract and related organs emphasizes the relative importance of culturing these tissues in tracking down S. pullorum.

All cultures were made by placing a large (1 to 2 cubic centimeter) piece of the organ or a generous amount of its contents, if fluid, into

a large tube of tetrathionate broth, and plating to MacConkey's agar or SS agar, the former being the plating medium of choice. Dextrose, lactose, sucrose, and maltose were the sugars routinely used for identification.

SUMMARY AND CONCLUSIONS

A field survey regarding variant pullorum infection was made in 4 States: Indiana, Kentucky, Minnesota, and Ohio.

One hundred seventeen birds were collected that reacted, with few exceptions, to variant or to polyvalent antigens but not to standard antigens. Seventy-five were subjected to bacteriological examination. S. pullorum was isolated from 26 of the 75; S. gallinarum fron one. Of 16 cultures submitted for typing, 15 were variants. Since 49, or about 65%, of the birds originally classed as variant infected by agglutination tests failed to reveal S. pullorum on autopsy, variant and polyvalent antigens appear inclined to yield non-specific reactions.

The isolation of S. pullorum from birds that were negative to standard antigen but positive to variant type antigen is evidence that the variant type of infection is present in this country. There were 24 such birds (Table 2). Variant infection, therefore, is a factor that is to be considered in control work. The extent of the variant type of infection was not indicated. The findings do not justify abandonment of present methods of control, partly because the survey was limited and also because in nearly 2/3 of the birds posted the S. pullorum organism was not isolated. The findings do indicate the desirability of the use of supplemental methods, that is, the use of polyvalent plate antigens or variant tube antigens whenever difficulties occur such as breaks in clean flocks or failure to reduce losses, and also the use of such antigens in the further surveys of the extent of variant infection. However, results of field tests with variant type antigen should be checked by careful bacteriological examinations.

REFERENCES

1. Younie, A. R.; Canad. J. Comp. Med., 5: 164, 1941.
2. Edwards, P. R. and Bruner, D. W.; Cornell Vet. 26: 318-324, 1946.
3. Edwards, P. R. and Bruner, D. W.; Proc. 50th Annual Meeting of the U. S. Livestock Sanitary Assn., 1946.
4. Wright, M. L.; Proc. 16th Annual Meeting of the Northeastern Conference of Laboratory Workers in Pullorum Disease Control, 1944.
5. Report of the Chief of the Bureau of Animal Industry, Agricultural Research Administration, 1947.

TABLE 1

POSITIVE REACTIONS OF PULLORUM REACTOR FLOCK TO STANDARD AND POLYVALENT ANTIGENS

Test No.	Date	No. Tested	Whole Blood Plate Test				Tube Tests			
			Standard Antigen		Polyvalent Antigen		No. Tested	Standard Antigen		Polyvalent Antigen	
			No.	Pct.	No.	Pct.		No.	Pct.	No.	Pct.
1	10-10-47	117	16	13.6	115	98.3					
2	11-5-47	108	22	20.3	95	87.9					
3	1-22-48	51	24	47.1	47	92.1	50	27	54.0	50	100
4	2-27-48	40	24	60.0	38	95.0					
5	5-6-48	32	14	43.8	31	96.9	31	21	67.7	28	90.3
	TOTALS	348	100	28.7	326	93.6	81	48	59.2	78	96.3

TABLE 2

TESTS AND POSTMORTEM RESULTS OF VARIANT PULLORUM REACTIONS

Lot. No.	Date	No. Killed	S. Pull. Recovered	First (Field) Plate Agglutionation Reaction of Positive Birds			
				Standard Antigen		Polyvalent Antigen	
				Pos.	Neg.	Pos.	Neg.
1	10-10-47	4	4	0	4	4	0
2	10-17-47	6	5	0	5	5	0
3	11-18-47	11	2	0	2	2	0
4	12-9-47	10	2	0	2	2	0
5	1-7-48	17	1	0	1	1	0
6	1-14-48	14	3	0	3	3	0
7	11-15-47 to 4-19-48	Died 12	9	2	7	9	0
	TOTALS	75	26	2	24	26	0

TABLE 3

FREQUENCY OF VARIANT PULLORUM ISOLATIONS

Organ	No. of Isolations	Percent of Birds from which recovered	Isolated from one organ only
Ovary	20	86.9	9
Gall Bladder	12	52.1	1
Pancreas	9	39.1	
Spleen	9	39.1	
Liver	8	34.8	
Duodenum	7	30.4	
Oviduct	7	30.4	
Proventriculus	6	26.0	
Crop	6	21.7	
Pericardial sac	6	21.7	
Lung	3	13.0	1
Kidneys	2	8.7	

TOTAL ISOLATIONS 95

TOTAL Pullorum positive birds - 26

TABLE 4

DISTRIBUTION OF PULLORUM CULTURES

	No. of Isolations	Percent of Isolations
Digestive tract:		
Crop	6	
Bile	12	
Proventriculus	6	
Pancreas	9	
Duodenum	7	
TOTALS	40	42
Reproductive tract:		
Ovary	20	
Oviduct	7	
TOTALS	27	28
Miscellaneous		
Pericardial fluid	6	
Lung	3	
Liver	8	
Spleen	9	
Kidney	2	
TOTALS	28	29